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(71) Applicant:

000006091

Meiji Seika Kaisha, Ltd.

4-16 Kyobashi 2-chome, Chuo-ku

Tokyo-to

(72) Inventor:

Megumi Yamagishi

c/o Biological Sciences Research Laboratories

Meiji Seika Kaisha, Ltd.

5-3-1 Chiyoda

Sakado-shi, Saitama-ken

(72) Inventor:

Naomi Osakabe

c/o Biological Sciences Research Laboratories

Meiji Seika Kaisha, Ltd.

5-3-1 Chiyoda

Sakado-shi, Saitama-ken

(72) Inventor:

Atsushi Nagaki

c/o Biological Sciences Research Laboratories

Meiji Seika Kaisha, Ltd.

5-3-1 Chiyoda

Sakado-shi, Saitama-ken

(72) Inventor:

Toshio Takizawa

c/o Biological Sciences Research Laboratories

Meiji Seika Kaisha, Ltd.

5-3-1 Chiyoda

Sakado-shi, Saitama-ken

(72) Inventor:

Takashi Ozawa

c/o Faculty of Agriculture

Nagoya University

Furo-cho, Chikumi-ku

Nagoya-shi, Aichi-ken

(54) [Title of the Invention] Health Beverage and Food Products That Contain an Anticarcinogenic Substance

(57) [Abstract] This invention provides beverage and food products that have anticarcinogenic action and safety in which cacao beans are the raw material. [Structure] Health beverage and food products characterized in that they contain an anticarcinogenic substance that is extracted from cacao beans using ethanol.

[Claims]

[Claim 1] Health beverage and food products characterized in that they contain an anticarcinogenic substance that is extracted from cacao beans using ethanol.

[Claim 2] Health beverage and food products as described in Claim 1 in which the anticarcinogenic substance is extracted from cacao beans using ethanol at a water-to-ethanol ratio of 60 : 40 - 0 : 100.

[Claim 3] Health beverage and food products as described in Claim 1 in which the anticarcinogenic substance is a crude polyphenol that is obtained by further purification of the ethanol extract.

[Detailed Description of the Invention]

[0001]

[Technological field of the invention] This invention relates to health beverage and food products that contain a natural carcinogenic substance, and, in detail, it relates to health beverage and food products that contain an anticarcinogenic substance that is extracted from cacao beans.

[0002]

[Prior art] Cacao (*Theobroma cacao*) is an evergreen tree of the family Sterculiaceae that is a product of the moist zone of tropical America. It is a fruit of 15 to 30 cm in length and of on the order of 10 cm in diameter. It has been cultivated for over 2000 years and has become acclimated. The cacao beans in the fruit are used as the raw material of such beverage and food products as chocolate and cocoa. It has been reported that the alkaloids in the cacao bean have physiological actions such as cardiac stimulation and a diuretic action. It is further known that the cacao beans contain polyphenols that have an antioxidant action. However, there have been no reports of anticarcinogenic action.

[0003] Even at present, with remarkable progress having occurred in medicine, there is a tendency toward an increase in cancer patients, with cancer being in first place as a cause of death among Japanese. As we move further into an aging society, there is the possibility that the increase will be greater than it has been up to the present. On the other hand, research on the development of cancer is proceeding and the facts that our dietary habits and living environment account for many of the causes of cancer and the mechanisms of its occurrence are being clarified.

[0004]

[[Problems the invention is intended to solve] Accordingly, searches for anticarcinogenic substances in foods, clarification of their mechanisms of action and research on methods of manufacture are proceeding not only from the standpoint of medicinal drug products but also from that of improving dietary habits for the purpose of preventing the occurrence of cancer. The development of effective health beverage and food products is anticipated.

[0005]

[Means for solving the problems] The inventors conducted intensive and repeated research for the purpose of solving the aforementioned problems. As a result, they confirmed that there is an anticarcinogenic substance of superior physiological effectiveness in cacao beans, which are the principal raw materials of chocolate and cocoa and they perfected this invention on the basis of this knowledge. Specifically, this invention provides health beverage and food products that are characterized in that they contain an anticarcinogenic substance that is extracted from cacao beans using ethanol.

[Mode of execution of the invention] The method whereby the anticarcinogenic substance of this invention is manufactured from cacao beans is extraction with ethanol, which is not harmful to the human body. The cacao beans that serve as the raw materials are used in various forms such as cacao shells, cacao ribs, cacao mass and defatted cacao mass. Defatted cacao mass is preferable in that the extract can be made into a powder. Defatted cacao mass can be obtained by removing the oil from the cacao mass, from which the part of the exodermis called the cacao shell is removed from the cacao bean. Removal of the oil may be effected by known methods.

[0007] Extraction is performed by adding 100 to 800 parts by weight, and, preferably, 400 to 700 parts by weight, of ethanol solution to 100 parts by weight of cacao beans and by stirring the mixture at normal temperature for several hours to 48 hours, and, preferably, for approximately 24 hours. The ethanol solutions that can be used are solutions in which the water to ethanol ratio is 60 : 40 - 0 : 100, and, preferably, 30 : 70 - 0 : 100.

[0008] The ethanol in the extraction solution, which is obtained by filtration, is removed under decreased pressure, after which it is made into powder by freeze-drying or drying under reduced pressure. As required, the extracted matter may be further purified by chromatography. As an example, open column chromatography (chromatography using Diaion HP2MG, which is one of the most commonly used methods at present) is performed and crude polyphenol, which is the principal component of the anticarcinogenic substance, can be obtained. The anticarcinogenic substance that is obtained in this way may be used as a concentrated solution or a powder depending on the intended use of the beverage or food product.

[0009] The anticarcinogenic substances in the food product, of course, may be ingested individually or may be ingested as a mixture of several components. A superior effect may frequently be manifested by a synergic action among the anticarcinogenic substances. Consequently, it is necessary to consider the manifestation of an overall effect of multiple components in the food products rather than studying the effects of the individual components of the anticarcinogenic substance. On this basis, the inventors evaluated the anticarcinogenic action of the extract obtained from the cacao beans taking as the index the inhibitory action on mutagenesis of the carcinogenic substance and of mutagenic substances which act as the triggers of development of cancer in cells.

[0010] A well-known method of screening the mutagenicity of chemical substances is the Ames method using *Salmonella typhimurium*. As the number of substances that are detected as being mutagenic and that are confirmed to be carcinogenic increases, this method has come into wide use as a simple procedure for detecting carcinogenic substances. It has become possible to create states in which mutagenesis occurs and to evaluate the inhibitory action of substances on mutagenicity, i.e., inhibitory action on carcinogenesis, by using substances of which the carcinogenicity has been confirmed in advance in this experimental system (T. Osawa, et al., *Environmental and Molecular Mutagenesis*, 18, 35-40, 1991). As a result of performing mutagenicity tests by this modified form of the Ames method, it was found that there was a strong anticarcinogenic action in the cacao extract.

[0011] The healthy beverage and food products in this invention are beverage and food products that contain anticarcinogenic substances that are extracted from cacao beans using ethanol. Because this substance is nontoxic to the human body, when it is actually ingested as a health beverage and food product, it should be used in an amount on the order of 1 to 1000 mg/kg of body weight/day, and, preferably, of 10 to 500 mg/kg of body weight/day. Taking the characteristics, flavor and economic factors of various beverage and food products into consideration, the quantity added should be on the order of 0.1 to 20%, and, preferably, 0.2 to 10%.

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[0012] The health beverage and food products of this invention can include, of course, beverage and food products such as chocolate and cacao that use cacao beans as the principal raw materials, and, as long as they are products to which the extract of this invention is added, starch food products such as bread, biscuits and noodles or beverage and food products such as candy, processed cow's milk and chocolate can be used. They may be compounded in appropriate stages of manufacture and processing depending on the characteristics of the various beverage and food products and the objectives.

[0013]

[Examples] We shall now describe this invention in specific terms by presenting examples. However, this invention is not limited by these examples.

[0014] Example 1. Method of manufacture of carcinogenesis inhibiting substances

500 parts by weight of 80% ethanol was added to 100 parts by weight of defatted cacao mass, stirring and extraction were performed at room temperature for 24 hours and the extraction solution was filtered, after which it was concentrated under reduced pressure. The concentrated solution that was obtained was freeze-dried and a cacao extract (80% ethanol extract) was obtained. Next, 30 ml of supernatant obtained from centrifugation of the concentrated solution that was obtained was applied to a Diaion HP2MG column (size, 35 mm i.d. x 310 mm; manufactured by Mitsubishi Chemical Company) and the alkaloids were eluted and removed with an eluent comprised of purified water and ethanol in a ratio of 80 to 20. An active fraction having a high polyphenol content was obtained with an eluent comprised of purified water and ethanol in a ratio of 20 to 80. 30 ml of this fraction was applied to a silica gel column (size, 35 mm i.d. x 310 mm) and chromatography was performed. Elution was performed with a mixed solution of chloroform of methanol and an active concentrated fraction was obtained in an eluent comprised of chloroform and ethanol in a ratio of 60 to 40. Figure 1 shows a synopsis of the purification method. The active fraction and the active concentrated fraction were used in the experiments after freeze-drying.

[0015] Example 2. Evaluation of carcinogenesis inhibitory activity by antimutagenicity tests

50 μ l of test substance, 100 μ l of mutagenic substance, 300 μ l of the metabolic activation enzyme S-9 mix (a mixture of S-9 for use in antimutagenicity tests and of cofactor I for use in the Ames test (manufactured by the Oriental Yeast Company)) and 100 μ l of Salmonella typhimurium TA98 bacterial solution were added to a test tube and were thoroughly mixed, after which the mixture was incubated for 20 minutes at 37°C. After incubation, 2.5 ml of soft agar solution (obtained by adding one-tenth the volume of 0.5 M histidine-biotin solution to 2.4 g of agar, 12.0 g of NaCl and 400 ml of water) was immediately added and the substances were thoroughly mixed, after which the mixture was introduced into minimum glucose agar plate culture medium. The materials were allowed to stand for 2 days at 37°C, after which the number of back mutated colonies that had grown was counted. The active fraction and the active concentrated fraction that were obtained in Example 1 were used as the test substances. Trp-P-2, which is a heterocyclic amine originating from food products of which the carcinogenicity had been confirmed, was used as the mutagenic substance. Trp-P-2 itself, which is a heat denatured product of tryptophan, does not have carcinogenicity. However, it is known that it is absorbed into the body and is metabolized by liver enzymes, whereby it is transformed into an active substance having strong carcinogenicity, with mutations being induced by bonding with DNA.

[0016] The results of the test are shown by the number of colonies (number of back-mutated colonies) in which mutations have been brought about. As a result, as shown in Table 2, the number of back-mutated colonies was decreased in a dose-dependent pattern by adding the test substances. The mutation

Inhibition rates were 28% and 34%, respectively, with 100 µg/plate and 200 µg/plate of active fraction and 48% and 72%, respectively, with 100 µg/plate and 200 µg/plate of active concentrated fraction, it being confirmed that an carcinogenesis inhibiting action was found.

[0017] Example of Manufacture 1

Chocolate was made by the following formulation in accordance with a standard method using the cacao extract obtained in Example 1.

Cacao mass; 50 parts

Cacao butter; 50 parts

80% ethanol extract; 5 parts

Sugar; 95 parts

Cow's milk; 2 parts

Shortening; 10 parts

[0018] Example of Manufacture 2

A lozenge was made by the following formulation in accordance with a standard method using the cacao extract obtained in Example 1.

Granulated sugar; 85 parts

Concentrated fruit juice; 5 parts

Citric acid; 6 parts

Fragrance; 2 parts

80% ethanol extract; 1 part

Gelatin; 1 part

[0019] Example of Manufacture 3

A cookie was made by the following formulation in accordance with a standard method using the cacao extract obtained in Example 1.

Wheat flour; 440 parts

Sugar; 240 parts

Margarine; 200 parts

Whole egg powder; 70 parts

80% ethanol extract; 20 parts

Vanilla essence; 2.5 parts

Water; 130 parts

[0020] Example of Manufacture 4

A cocoa beverage was made by the following formulation in accordance with a standard method using the crude polyphenol (active fraction) obtained in Example 1.

Crude polyphenol (active fraction); 0.5 parts
Cocoa powder; 2 parts
Sugar; 4.5 parts
Defatted powdered milk; 0.5 parts
Emulsifying agent; 0.1 parts
Table salt; 0.05 parts
Vanilla flavoring; 0.05 parts
Treated water; 92.3 parts

[0021]

[Effect of the Invention] By means of this invention, health beverages and foods having a carcinogenesis inhibiting action can be provided using cacao beans, which have long been used as a food product, as the raw material. Cacao beans have been used as a food product since before the Christian era and large quantities are consumed at present throughout the world. Thus, their safety has been confirmed. On the basis of the foregoing facts, the health beverage and food products of this invention, by their consumption, can provide a superior means of preventing cancer and of preventing various types of diseases and of preventing aging which are said to be induced by damage to DNA. Thus, they are of extremely great significance.

[Brief Explanation of the Figures]

[Figure 1] This is a flow chart showing the method of purification of defatted cacao mass extract.

[Figure 2] This is a graph showing the inhibitory action of mutagenesis of the defatted cacao mass extract as ascertained by antimutagenicity tests.

Figure 1. Method of Purification of Carcinogenesis Inhibiting Substances From Defatted Cacao Mass

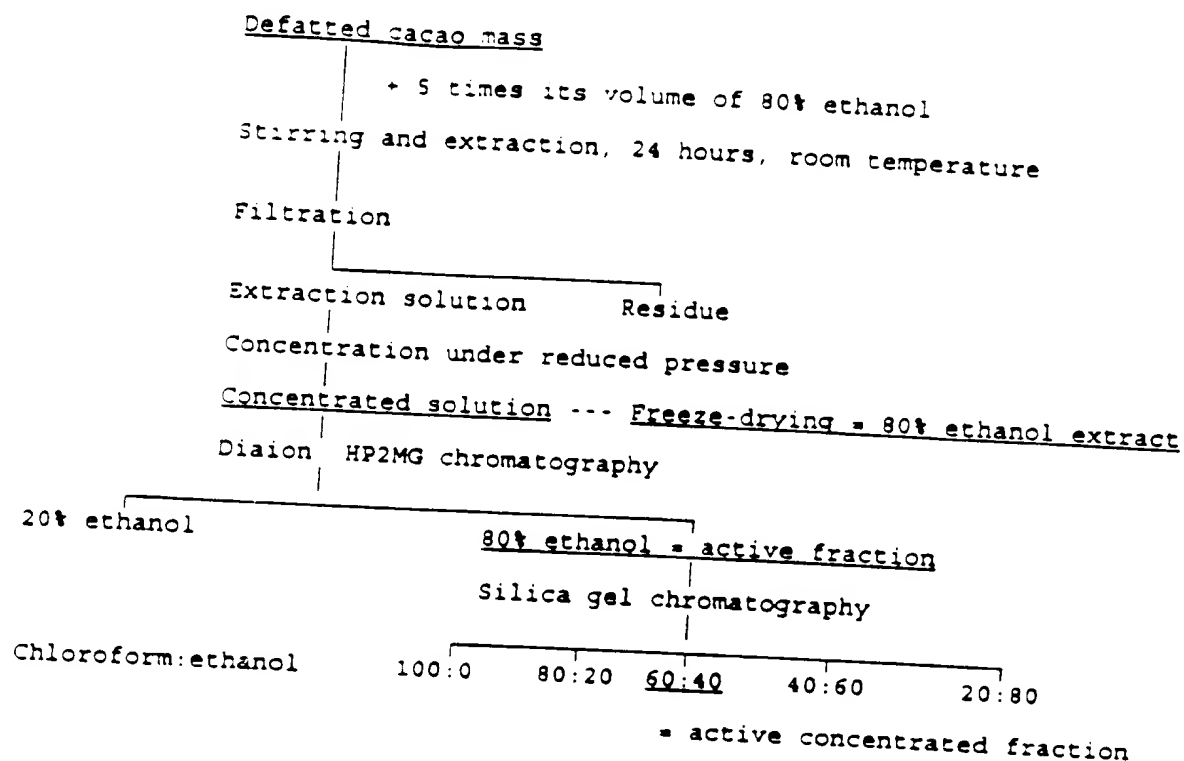
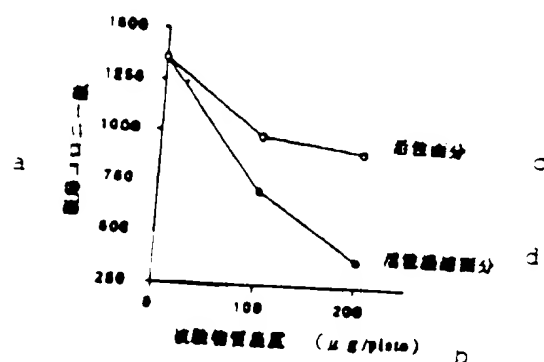


Figure 2. Inhibitory Action on Mutagenesis of Defatted Cacao Mass Extract



KEY TO TRANSLATION:

- [vertical axis]:
a. Number of back-mutated colonies
[horizontal axis]:
b. Concentration of test substance ($\mu\text{g/plate}$)
[inside figure, top to bottom]
c. active fraction
d. active concentrated fraction

[Note: Matter on page (5) of the Japanese patent included in first page heading]